D. Lawrence
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Hunter	Att'y Docket:	1118/174
Serial No:	09/710,082	Art Unit:	1743
Date Filed:	November 10, 2000	Examiner:	Soderquist, A.
Invention:	METHODS FOR PERFORMING MICROASSAYS		

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CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to Commissioner for Patents, Washington, D.C. 20231 on April 14, 2003.

Samuel J. Petuchowski

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Commissioner for Patents
Washington, DC 20231

Declaration of Dr. Colin J. H. Brenan
(37 CFR §1.132)

Dear Sir:

I hereby declare that:

1. My name is Dr. Colin J. H. Brenan. I am co-founder and CEO of BioTrove, Inc., a Cambridge, Massachusetts-based company specializing in advanced biotechnologies and bioassay equipment.
2. I hold B.Sc (Honors Physics), M. Eng. (Electrical) and Ph.D. (Biomedical Engineering) degrees from McGill University, and have over thirteen years experience in technical program management and product development including

eight years at MPB Technologies Inc. and five years at the Department of Mechanical Engineering at the Massachusetts Institute of Technology. I am the inventor of a multiple image mode confocal optical microscope (MIMCOM), microfluidic systems for tissue-based biosensors, semi-autonomous microsurgical devices and ultra high throughput biomolecular discovery technologies. I am the principal author of over forty-five publications and hold six patents in the areas of communications, microfluidic systems and optical technologies.

3. This Declaration is submitted in support of a U.S. Patent Application, Serial No. 09/710,082 (the "Application"), for an invention by Dr. Ian Hunter.
4. More particularly, this Declaration addresses issues bearing upon a determination of whether the invention that is the subject of claims pending in the Application would have been obvious to a person of ordinary skill in the art as of the date, January 5, 1999, when a Provisional Patent Application, upon which the Application is based for purposes of priority, was filed.
5. My company, BioTrove, Inc., was founded in 1997 as Advanced Instrumentation Systems. The BioTrove team, currently numbering 21 employees, includes engineers within the disciplines of mechanical, chemical, software, biomedical and optical engineering and scientists with expertise in diversity biology, enzymology, toxicology, analytical chemistry, materials and surface science. BioTrove is dedicated to delivering dramatic increased in the density and rate at which chemicals and materials may be stored, manipulated and tested for desired properties. Areas of applications for BioTrove's technology include drug discovery, protein engineering, genomics, proteomics, biosensors, chemical engineering and materials sciences.
6. On May 11, 2001, BioTrove, Inc. obtained an exclusive license to the invention of Professor Ian Hunter that is the subject matter of the present Application, in order to obtain the advantages of massive parallel assays of liquid samples retained at high spatial density. Since July, 2002, BioTrove has employed the claimed method, and a cognate product under the tradename Living Chip™, in performance of a contract to screen 500,000 compounds in conjunction with 3 protein targets. The Living Chip™ technology enables rapid initiation and

analysis of 10,000 to 1,000,000 reactions simultaneously. The Living Chip™ consists of a plate of high density, micromachined through-holes providing the functionality of a microtiter plate with the density of a micro-array. BioTrove's mid-term goal is to develop a platform to screen as many as one billion samples per day in volumes of less than 50 nanoliters each. BioTrove plans to develop methods for synthesizing arrays of molecules directly on the Living Chip™.

7. The Living Chip™ technology, subject to the current claims, has also enabled BioTrove to obtain close to $2M in funding from the National Institute of Standards and Technology (NIST) under its Advanced Technology Program (ATP) under which innovative proposals are funded on the bases of peer review of potential economic benefit to the nation. The nexus of the funding to the claimed elements is apparent from the ATP Project Brief which is appended hereto as Exhibit A.
8. Prior to licensing the subject Application, BioTrove conducted an exhaustive evaluation of the then-state-of-the-art of art of high-throughput handling of liquid samples for the express purpose of streamlined screening, particularly of libraries of reagent compounds.
9. Then-available technology for parallel processing and assay of liquid samples was limited to well plates, with densities up to 1,536 wells per plate, although plates of that well density had not achieved significant market penetration at the time, owing to difficulties of liquid handling in such densities that are attendant to the plate format. Well densities limited the possible rate of early drug development steps, suggesting the immense economic value of a massive increase in the number of samples that could be handled in a specified period of time. Since there is a time overhead involved in the loading and analysis of a plate, an increase in density translates to an increase in overall sampling rate. Moreover, the use of smaller sample sizes decreases target, compound, and reagent consumption requirements.
10. While the economic advantages of higher sample densities to achieve higher assay throughput had long been known, the industry had failed, until the time of the present invention, to fill that need and to achieve the anticipated advantages.

The state of the industry, as of May, 2001, is summarized in Rubenstein & Coty, *High-Throughput Screening: Redefining the Mission*, (2d Ed., Drug & Market Development Publications, 2001; pp. i-iv, and 1-1 – 1-14, appended hereto as Exhibit B). According to Rubenstein & Coty, "The most important consumable item in most high-throughput screening assays is the microtiter plate, or microplate." (*Ibid.*, p. 1-7.)

11. The problems limiting increased density of microtiter plates included volume reproducibility in dispensing samples into the plates; evaporation from the plates, electrostatic repulsion of droplets, ambiguity in the position of droplets limiting the reproducibility of mixing steps, and the trapping of air bubbles between the liquid samples and the bottoms of the microwells.

12. While meshes were known that could provide support for a single fluid held across the openings of the mesh by surface tension, it was not apparent in the art how distinct samples might be supported by such a mesh, since, whereas cross-talk within a homogeneous sample might be tolerable, cross-talk among distinct samples is a critical deficiency.

13. BioTrove, Inc. employs inventive features encompassed in the requirements of the presently pending claims. In particular, the BioTrove Living Chip™ provides a customer with a platen having two substantially parallel planar surfaces, an inner layer of hydrophilic material, two outer layers of hydrophobic material coupled to opposite sides of the inner layer, and a two-dimensional array of a plurality of addressable through-holes. The through-holes are substantially perpendicular to the planar surfaces and are characterized by an areal density of at least 1.6 through-holes per square millimeter, allowing the customer, paraphrasing the words of the first independent claim, to:

    a. load a first sample into a first set of through-holes of the two-dimensional array, the first sample being a liquid;

    b. retain the first sample in the first set of through-holes by surface tension;

    c. add a second sample into a specified through-hole, the specified through-hole having at least one adjacent through-hole containing a sample other

than the second sample, the specified through-hole further coinciding with one of the first set of at least one of the though-holes thereby permitting a reaction between the first sample and the second sample; and

d. characterize the reaction in the through-hole in terms of the specified properties.

14. The Living Chip™ product has achieved substantial market recognition and commercial success. Several large pharma and biotech firms, in a market survey that was conducted on behalf of my firm, have identified advantages of the Living Chip™ product in fulfilling unmet needs of high throughput, lower evaporation, and lower sample volume, all deriving from the claimed features of high sample density with distinct samples in adjacent through-holes with surface treatment measures adopted to counteract unintended liquid mixing.

15. While the Living Chip™ product is not compatible with existing assay apparatus formats such that its adoption in the industry entails significant retooling costs, the Living Chip™ product has been adopted by 3 current major customers, each from among the Top-10 pharma corporations, because of the benefits deriving from the claimed features of the present invention.

16. I declare that all the statements made here are of my own knowledge and that I believe them to be true; and further these statements were made with the knowledge that willful false statements are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

Dr. Colin J. H. Brenan

Dated: April 11, 2003

01118/00174 241118.1

ADVANCED TECHNOLOGY PROGRAM

NIST

Project Brief

Open Competition - Biotechnology (October 2000)

The Living Chip-Automated Microarray Technology For Homogeneous And Inhomogeneous Bioassays

Develop a novel modular system that manipulates microvolumes of liquids to conduct up to 100,000 individual biochemical assays simultaneously for high-throughput drug discovery and evaluation.

Sponsor: BioTrove, Inc. (formerly Advanced Instrumentation Systems)

620 Memorial Drive
Cambridge, MA 02139

- Project duration: 4/1/2001 - 3/31/2003
- Total project (est.): $2,988,368.00
- Requested ATP funds: $1,992,245.00

Antibodies produced by the immune system are useful tools for the discovery of new pharmaceuticals, but the latest analytical methods using antibodies are time consuming, expensive, and have problems that reduce the quality of the results. A faster, cheaper, and more reliable discovery method is envisioned by BioTrove, which plans to develop an automated technology platform (LivingChip{Tm}) that can perform up to 100,000 biochemical reactions in parallel using very small volumes of liquid. The system will have eight modules, the central one consisting of a stack of palm-sized plates, each containing an array of as many as 100,000 pinholes just two or three times the width of a human hair. Each pinhole in a plate mates with one in the next plate, so that the plates can be stacked to form continuous channels that form microreaction vessels. Volumes of reagents or other liquids ranging from 30 to 100 nanoliters can be selectively loaded into the channels in individual plates. When the plates are stacked, the various components mix and 100,000 individual reactions take place simultaneously. In the two-year project, BioTrove will develop a novel machining method to manufacture the plates out of various materials, such as glass and plastic, as needed for different applications. A central challenge is to fabricate the tiny pinholes with extremely tight tolerances at costs low enough to support commercial development. The other modules will include a camera for detecting fluorescent emissions from each

microchannel following a chemical reaction; a liquid dispensing and handling system; an assay station where the various modules come together; temperature and humidity controls; a plate transfer system; and a data analysis system. The ATP funding is needed to help BioTrove, a small startup, develop the prototype needed to obtain private financing. If successful, the new technology could reduce the cost of screening potential new drugs by one to two orders of magnitude, while also using smaller amounts of scarce chemical resources. The system would be 40 times faster than today's best assay systems, at one-third to one-half the capital cost. The system will be made available first to drug companies and diagnostic laboratories, and then, after further cost reductions, to point-of-care facilities such as hospitals. The system also could be used for materials discovery and combinatorial chemistry.

For project information:

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ATP Project Manager

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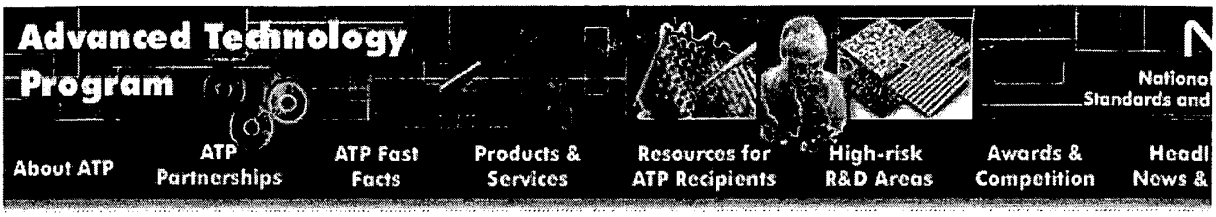

Overview of ATP

The Advanced Technology Program (ATP) bridges the gap between the research lab and the market place, stimulating prosperity through innovation. Through partnerships with the private sector, ATP's early stage investment is accelerating the development of innovative technologies that promise significant commercial payoffs and widespread benefits for the nation. As part of the highly regarded National Institute of Standards and Technology, the ATP is changing the way industry approaches R&D, providing a mechanism for industry to extend its technological reach and push out the envelope of what can be attempted.

Technology research in the private sector is driven by today's global, economic realities. The pace of technological change is faster than ever before, and victory goes to the swift. These realities force companies to make narrower, shorter-term investments in R&D that maximize returns to the company quickly.

The ATP views R&D projects from a broader perspective – *its bottom line is how the project can benefit the nation.* In sharing the relatively high development risks of technologies that potentially make feasible a broad range of new commercial opportunities, the ATP fosters projects with a high payoff for the nation as a whole – in addition to a direct return to the innovators. The ATP has several critical features that set it apart from other government R&D programs:

- ATP projects focus on the technology needs of American industry, not those of government. Research priorities for the ATP are set by industry, based on their understanding of the marketplace and research opportunities. For-profit companies conceive, propose, co-fund, and execute ATP projects and programs in partnerships with academia, independent research organizations and federal labs.
- The ATP has strict cost-sharing rules. Joint Ventures (two or more companies working together) must pay at least half of the project costs. Large, Fortune-500 companies participating as a single firm must pay at least 60 percent of total project costs. Small and medium-sized companies working on single firm ATP projects must pay a minimum of all indirect costs associated with the project.
- The ATP does not fund product development. Private industry bears the costs of product development, production, marketing, sales and distribution.
- The ATP awards are made strictly on the basis of rigorous peer-reviewed competitions. Selection is based on the innovation, the technical risk, potential economic benefits to the nation and the strength of the commercialization plan of the project.
- The ATP's support does not become a perpetual subsidy or entitlement – each project has goals, specific funding allocations, and completion dates established at the outset. Projects are monitored and can be terminated for cause before completion.

The ATP partners with companies of all sizes, universities and non-profits, encouraging them to take on greater technical challenges with potentially large benefits that extend well beyond the innovators – challenges they could not or would not do alone. For smaller, start-up firms, early support from the ATP can spell the difference between success and failure. To date, more than half of the ATP awards have gone to individual small businesses or to joint ventures led by a small business. Large firms can work with the ATP, especially in joint ventures, to

develop critical, high-risk technologies that would be difficult for any one company to justify because, for example, the benefits spread across the industry as a whole.

Universities and non-profit independent research organizations play a significant role as participants in ATP projects. Out of 642 projects selected by the ATP since its inception, well over half of the projects include one or more universities as either subcontractors or joint-venture members. All told, there are more than 160 individual universities participating in ATP projects.

ATP awards are selected through open, peer-reviewed competitions. All industries and all fields of science and technology are eligible. Proposals are evaluated by one of several technology-specific boards that are staffed with experts in fields, such as biotechnology, photonics, chemistry, manufacturing, information technology, or materials. All proposals are assured an appropriate, technically competent review even if they involve a broad, multi-disciplinary mix of technologies.

The ATP accepts proposals only in response to specific, published solicitations. Notices of ATP competitions are published in Commerce Business Daily. You may also request to be placed on a mailing list to receive notification of ATP competitions and other events by calling the ATP automated hotline (1-800-ATP-FUND) or by sending e-mail to atp@nist.gov. The ATP Proposal Preparation Kit may be requested at any time. In addition to the necessary application forms, the kit includes a thorough discussion of the ATP goals and procedures as well as useful guidelines in the preparation of a proposal. Further information can also be found on the program's web site.

Hotline 1-800-ATP-FUND (1-800-287-3863)
Email atp@nist.gov
Fax: (301) 926-9524
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D&MD Reports

High-Throughput Screening

Redefining the Mission

2nd Edition

by Ken Rubenstein, Ph.D. & Cynthia Coty

Published May 2001
Report #9032
260+ Pages • 41 Exhibits • 20 Company Profiles

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Executive Summary

HIGH-THROUGHPUT SCREENING:
REDEFINING THE MISSION

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1.0 Executive Summary

1.1 Introduction

The screening of chemical compounds for pharmacological activity has been ongoing in various forms for at least forty years. The screening paradigm says that when a compound interacts with a target in a productive way, that compound then passes the first milestone on the way to becoming a drug. Compounds that fail this initial screen go back into the library , perhaps to be screened later against other targets.

The perceived needs of the pharmaceutical industry have changed with time and driven marked changes in the industry, both in terms of its business structures and its scientific approaches to drug discovery and development. In response, screening methodologies have also improved with time, both in terms of throughput and the amount of information to be derived from the screen. Advances in assay and instrument technologies have provided the means necessary to address these evolving needs.

Big pharma has relied to a large extent on blockbuster drugs to maintain growth. Many of the largest companies currently face pipeline gaps and must resort to heroic improvisations as mainstay products go off-patent. New technologies and perspectives, culminating in what has come to be called the industrialization of drug discovery, are helping to add predictability and manageability to the pharmaceutical enterprise. Major shifts in the market environment are prompting innovative responses from big pharma, including the expectation that drug development will keep pace with the rapid expansion of new medical knowledge and technology advances. A trend toward products with added information content may well drive companies toward greater disease specialization with franchises based on packages of goods and services.

Long-term industry responses to a changing environment leave a gap during which companies must find interim solutions to maintain profits at acceptable levels. Companies are pressured to increase rates of new product introductions and profitability, as managed care groups erode prices and volumes, and governments call for more restrictive terms of exclusivity and cost controls. These and other factors have led to substantial increases in R&D costs as a percentage of sales. Large expenditures in time and costs required for companies to introduce new drugs, together with a large project attrition rate, have led to the perception of an innovation deficit. Companies on average introduce less than one drug per year, while between two and three drugs are required to maintain profits at acceptable levels. Indications of progress against the deficit

exist, but success is not yet assured. Mergers provide interim solutions, but scientific and technological advances promise more permanent results.

The report considers the elements of high-throughput screening technology by first placing the field in the overall context of the needs and pressures on the pharmaceutical industry and their suppliers, offers a definition of high-throughput screening, and discusses the origins and evolution of its current and emerging technologies. Turning next to a consideration of market factors, the report discusses current bioanalytical technologies, the changing nature of drug discovery, and the relevance of high-throughput screening in today's pharmaceutical industry.

The current status of high-throughput screening technology is delineated, first considering classical approaches, next the assay downsizing movement, the status of robotics and automation, and finally the role of microfluidics. In viewing the status of high-throughput screening research, the report considers key publications in the field, funding sources for relevant research, and the organization of the high-throughput screening research community. A subsequent sections views specific product offerings and their categorization.

In a section on business aspects, the report considers strategies of major players in the categories of pharmaceutical companies and technology platform companies. A discussion of collaborations and deals is followed by a market analysis in which current and projected sales for various product and service segments are considered. Following an extensive company profile section, the report concludes with a consideration of future trends in high-throughput screening.

Information for this report is derived from both primary and secondary sources. A thorough survey of company literature, trade journals, press releases, on-line articles, scientific journals, and the like generated a base of information, which was expanded through discussions and interviews with participants in the field, both pharmaceutical companies and equipment/reagent suppliers.

1.2 Elements of High-Throughput Screening Technology

High-throughput screening is a key link in the chain comprising the industrialized drug discovery paradigm. Today, many pharmaceutical companies are screening 100,000-300,000 or more compounds per screen to produce approximately 100-300 hits. On average, one or two of these become lead compound series. Larger screens of up to 1,000,000 compounds in several

months may be required to generate something closer to five leads. Improvements in lead generation can also come from optimizing library diversity. Limited success has been achieved to date in this realm.

High-throughput screening is perhaps most accurately understood as one stage in an evolving process. Since the 1980s, improvements in screening technologies have resulted in throughputs that have increased from 10,000 assays per year to current levels, which can approach ultra-high-throughput screening levels of more than 100,000 assays per day. High-throughput screening is evolving not only as a discrete activity, but as a perspective that is expanding backward toward target identification and validation and forward to converting assay hits to qualified leads via information generated either within screens or through downstream, high-throughput ADME (absorption, distribution, metabolism, and excretion) and toxicity testing.

In terms of definition, high-throughput screening can be considered the process in which batches of compounds are tested for binding activity or biological activity against target molecules. Test compounds act as inhibitors of target enzymes, as competitors for binding of a natural ligand to its receptor, as agonists or antagonists for receptor-mediated intracellular processes, and so forth. High-throughput screening seeks to screen large numbers of compounds rapidly and in parallel. Yet in another sense, high-throughput screening is an evolving process that is today a discrete activity and may tomorrow become more highly integrated into a rapidly changing drug discovery paradigm.

Positive high-throughput screening results are usually called hits. Compounds resulting in hits are collected for further testing in which, for example, the potency of an enzyme inhibitor or the binding affinity of a ligand for a receptor may be determined. After this second level of triage, hits become lead compounds. Further synthesis may then be required to provide a variety of compounds structurally related to the lead. These sub-libraries must then be screened against targets in order to choose optimal structures. At this stage, some basic indicators of toxicity or bioavailability may be considered in an attempt to eliminate potential failures as early in the discovery process as possible.

1.3 Market and Technology Factors Underlying High-Throughput Screening

High-throughput screening was formed from a confluence of market-driven needs within the pharmaceutical industry and technological strategies originated within the *in vitro* diagnostics

industry. This section examines the pharmaceutical industry market environment that has given rise to the need for the industrialization of research and the technology legacy that is finding a potential outlet for its creative energies.

A convergence of several trends is promising to yield a highly effective new paradigm for drug discovery. While the exact nature of the new paradigm is not yet clear, its outlines are starting to emerge from the mist. Trends include: the influence of genomics on increasing both the quantity and quality of new drug targets; advances in combinatorial organic synthesis to increase both the quantity and quality of compound libraries; the influence of high-throughput screening in providing an increased supply of new lead compounds; and the enhanced use of bioinformatics for process integration.

High-throughput screening borrows heavily from methods and equipment designed originally for biomedical research applications and later for *in vitro* diagnostic assays. Ligand-receptor assays originated with the Nobel-prize winning invention of radioimmunoassay during the 1960s. The early 1970s also saw the introduction of homogeneous technologies in which no physical separation of components was necessary in order to read a result. Homogeneous enzyme immunoassay and fluorescence polarization were used during the 1970s and 1980s to commercialize certain *in vitro* diagnostic assays. FRET (fluorescent resonance energy transfer) and time-resolved fluorescence assays were also developed in a clinical context. There is new awareness that high content screening (HCS) can help improve the screening process and overall discovery timeline. These assays can deliver multiple data points per well, and can often be achieved using cell based assays.

Microfluidic technologies, as practiced by development-stage companies such as ACLARA and Caliper, were originally intended for diagnostic application, particularly in the area of immunodiagnostics. However, shifts in the diagnostic market, due to forces similar to those imposing needs for research cost reductions and efficiency increases in the pharmaceutical industry, have led to decreased emphasis on the application of new technologies to diagnostics and increases in their application to drug discovery.

The pharmaceutical industry currently has a pressing need for improvement in high-throughput screening technology. Although the industry has a seemingly insatiable appetite for new lead compounds, it is also under continuing pressure to reduce the costs of discovery and

development. Historically, the industry has shown its enormous potential both for the improvement of human health and the realization of significant profits in that process. Over the past three decades, this potential has propelled rapid advancement both in the valuation of large pharmaceutical companies and in the basic sciences that provide them with their technical foundation.

In the last half of the current decade, high-throughput screening instruments, assays, and services have emerged as a significant growth market. From the field's origins with home-brew tests and generic research instrumentation, high-throughput screening has become an increasingly sophisticated and important element in the armamentarium of the drug discoverer. The discovery process is currently on a steep growth curve both with respect to the number of targets and compounds to be screened and the complexity of the assays required. Increased numbers of targets and compounds call for greater parallelism and/or increased throughput in screens. Furthermore, the expense and scarcity of targets and compounds have driven a trend toward smaller assay volumes through miniaturization.

Manufacturers have risen to the occasion with a plethora of new offerings, including new assays, new formats, new technologies, and new instruments. Microfluidics technologies, for example, are being applied to the development of systems that consume sub-nanoliter quantities of reagents. New alternative technologies and systems come at a cost, and manufacturers are continually faced with assessing their true cost-benefit ratios.

1.4 Current Approaches to High-Throughput Screening

Recurrent themes in high-throughput screening are change and evolution. Modern screening has been dominated by 96-well microplate technology until a few years ago, when 384-well plates began their rise to prominence. Still higher density plates are now starting to see use. The past was dominated by filtration-based studies using radiolabels, while the present is increasingly dominated by methods requiring much less manipulation. Homogeneous scintillation proximity assays are giving way to a host of homogeneous fluorimetric assay technologies. Likewise, enzyme and immunological assays with colorimetric detection are giving way to the fluorimetric and luminometric counterparts. This section starts with a discussion of targets for screening assays, and then proceeds to discussions of current and emerging assay technologies and instruments. It focuses first on the cell biological background underlying the choices of targets for high-throughput screening. This large complex subject is treated at only its

simplest level to provide a sense for the general reader of the kinds of pharmacological considerations that form the basis for screening. The section then proceeds to discuss some of the more prevalent types of assays in use today.

The maintenance of homeostasis is heavily dependent on intercellular signaling. Many drugs bind to cell receptors, and this binding process triggers sequences of intracellular reactions that have profound effects on the organism. The natural signaling process involves synthesis and release of the signal molecule, transport to the target cell, binding to a cell receptor, an alteration of cellular metabolism or induction of gene expression, and removal of the signal molecule. The study of signal-transducing molecules is useful for determining the mechanism of a drug's action or its side-effects, and the study of signal pathways provides information that helps researchers identify new targets.

Cell surface receptors come in four varieties. GPCRs (G protein-coupled receptors) act when the ligand-binding activates a G protein (a signal transducer). The second class, ion-channel receptors, undergo a conformational change upon ligand binding that results in triggering a flow of ions, which triggers a change in membrane potential of the affected cell. A third class, tyrosine kinase-linked receptors, act when ligand binding results in the conversion of receptor monomers to dimers, which activate one or more cytoplasmic tyrosine kinases. Receptors in the fourth category are those that possess intrinsic enzymatic activity.

Early GPCR assays were done with filter-based methodologies. The SPA (scintillation proximity assay) provided a simplified alternative to the filtration methodology. Yet another step in the evolution of such assays involves using whole cells, either adherent to microwell surfaces or in suspension. Ligands tagged with fluorophores can then be detected *in situ* with either a digital imaging system or by reflectance fluorimetry. Protein-protein interactions, which are of interest in the context of screening for diverse classes of drugs, can be studied through the use of fusion molecules combining the proteins to be studied for interactions with reporter components. In a commonly used method, the yeast 2-hybrid assay, one of the two proteins is fused to the DNA-binding domain of a transcription factor and the other is fused to the activation domain of the same transcription factor.

Tyrosine kinase assays, very common subjects for high-throughput screening, are done with either heterogeneous or homogeneous technologies. Tyrosine phosphatases, important in

deactivating proteins containing phosphorylated tyrosines, are assayed in a reverse manner from tyrosine kinases. Protease inhibitors are of interest as potential drugs, and protease assays are therefore useful in high-throughput screening. A number of screens are based on measuring the concentration of analytes. Examples in this category include the interleukins, IL-6 and IL-8. The binding of proteins to DNA acts to stimulate gene transcription, a key step in many disease processes. Assays for such binding are commonly done by the classical gel shift technique.

Reporter gene assays are commonly used to indicate that a test compound has succeeded in activating the transcription of genes under the control of a particular promoter. The ligand-gated ion channels for sodium, calcium, and chloride form another category of receptors. Compounds that act on ion channels have been evaluated using bioassays that measure their effect on action potential in tissue preparations. Advances in electrophysiological measurements have led to the use of patch clamp analysis of currents derived from specific ion channels.

A great many technologies are currently employed for high-throughput screening. Their diversity is limited only by the inventiveness of pharmaceutical industry researchers and the companies serving them. Enzyme immunoassays can be run in a wide variety of formats for different purposes. Fluorescent labels, which are increasing rapidly in popularity, have several advantages over enzyme labels for ligand-receptor assays. Probably the most important of these is protocol simplicity. Time-resolved fluorescence immunoassay, in its heterogeneous manifestation, dates back more than a decade. Radioisotope-based assay methods are still used widely in spite of logistical and safety concerns. Scintillation proximity assay technology combines the advantages of radioisotopic labels and homogeneity.

The most important consumable item in most high-throughput screening assays is the microtiter plate, or microplate. Three sizes are in common use – 96, 384, and 1536-well plates. Microplates now come in a variety of formats, matched to particular applications. Variations are found, for example, in color and opacity for matching to various optical modalities. The diversity of materials and plate designs offers users tremendous flexibility in fulfilling their needs.

High-throughput requires automation, which comes in two varieties – modular or integrated. Another view of the same dichotomy might use the terms workstation versus robotic automation. Most robotic systems consist of modules tied together by a robotic arm equipped

with "fingers" to grasp and move plates from one place to another. A key difference between modular and robotic automation involves the degree to which human intervention is required.

A large number of new assay technologies have been put forth in recent years, primarily by established and development-stage companies seeking to gain or increase a share of the high-throughput screening market. The most important and impressive of the emerging *in vitro* technologies for high-throughput screening fall into the category of homogeneous fluorescence assays. These include fluorescence polarization, time-resolved fluorescence, fluorescence lifetime, FRET, TRET (time-resolved energy transfer), fluorescence correlation spectroscopy, microvolume fluorometry, homogeneous chemiluminescence, and electrochemiluminescence.

Cell-based assays have potential significant advantages over *in vitro* assays. In addition to gaining information on the binding of compounds to receptors or their effect on enzyme activity, it is possible with cell-based assays to collect information about compound effects on cell function. Emerging technologies are continually making cell-based assays more appropriate for high-throughput screening.

Miniaturization of high-throughput screening assays involves increasing the density of microwells in microplates while decreasing their volume. Microfluidic approaches abandon the microplate concept in favor of flow systems involving networks of channels with diameters on the order of tens of microns. Miniaturization from 96 to 384-well microplates is progressing rapidly, while further reduction to 1536 wells is the subject of much current investigation. Even higher-density systems are in development. Microfluidic systems present a significant step downward in the miniaturization process.

Caliper Technology's first LabChips™, using microfluidics for high-throughput screening, are currently under development and field testing in the context of a series of technology access programs. Caliper's major competitor, ACLARA BioSciences, has joined forces with PE Biosystems to develop the nMAS system, a high-throughput screening platform based on an instrument under development by PE and a series of LabCard™ chips under development by ACLARA.

One obvious corollary of the industrialization of drug discovery has been an explosion of data generated, especially from high-throughput screening of large combinatorial compound

libraries. A key informatics requirement for high-throughput screening involves capturing assay results from microtiter plates and storing it in a database. All large pharmaceutical companies have high-throughput screening databases in place. Spotfire, a supplier of research and development decision support systems to the life sciences industry, provides Spotfire Pro™ and Spotfire Structure Visualizer™, two integrated tools for visual data mining and interactive visualization of large data sets. MDL Information Systems offers Assay Explorer™, a tool that permits viewing data in a variety of formats. Oxford Molecular offers RS^3 Discovery HTS™, an Oracle client-server application designed to assist combinatorial chemists and screeners to register small molecules, define assays and protocols, track compounds through the screening process, and analyze screening results in several ways, including substructure searching.

1.5 High-Throughput Screening Products and Services

Considerable variety exists in the high-throughput screening products and services offered to pharmaceutical companies in the pursuit of increased quality and frequency of new drug leads and products. The nature and diversity of these products and services is changing rapidly as pharmaceutical companies respond to changing demands of an evolving drug discovery and development environment and as vendors sharpen their own skills at targeting offerings in an increasingly competitive market environment.

Instrument offerings fall into several categories. A group of companies provide either entire modular automated screening systems or subsets of such systems. Other companies produce workstations that provide specialized functionality. These may become fully automated systems with relatively minor enhancement. Instruments for homogeneous assays, for which chemistry precludes the need for extensive automation, fall in this category. Instrument manufacturers, especially those in the workstation category, are usually driven to use their installed instrument base as a franchise for selling consumable items. Viewing a selection of comprehensive high-throughput screening systems reveals two categories that might be termed first and second-generation entries. Many companies offer one or more modules that can be used to assemble complete high-throughput screening systems. Assay workstations can be used as stand-alone microplate readers or act as modules in high-throughput screening systems. As progress toward miniaturization has increased plate densities, the time required to read a plate has naturally moved upward to the point that reading can become a rate-limiting step for assay turnaround. CCD (charge-coupled devices) equipped with special telecentric optics provide the opportunity to read all wells in a plate simultaneously.

Assay kits are especially profitable items for suppliers since their primary value is in time-saving and information-providing content rather than in the cost of materials themselves. The extreme diversity of assays needed in high-throughput screening makes the provision of kits somewhat more complicated than in the diagnostics business with its much more limited scope of assays, yet manufacturers are responding to customer needs both with new kits themselves and with key reagents and tested protocols covering broader classes of assays. Perforce, much of the assay-related business activity must come through provision of assay development and consulting. Some of the newer homogeneous technologies, in particular, require that vendors assist customers in developing assays using proprietary components. A substantial number of companies offer high-throughput screening assay development services, while several companies offer comprehensive discovery services.

Bioinformatics is taking an increasingly important role in the drug discovery process and is especially critical for HTS because of the extremely large quantities of data generated by the screening process and the absolute necessity of capturing and using that data efficiently, accurately and easily. Making the screening process accessible in appropriate form for use by disciplines outside of the screening group is increasingly needed. These include interfacing with combinatorial and medicinal chemistry groups and providing software for the entire management of the drug discovery process.

1.6 The Business of High-Throughput Screening

High-throughput screening appears at first glance to be a relatively straightforward activity, a sort of numbers game. Screening more compounds against more targets per unit time should generate more hits, which should generate more leads, which should generate more products. Some industry participants accept this view, others do not accept it all, and most offer provisos. No contemplation of high-throughput screening can be considered complete without recognition of two key factors: (1) drug discovery is in the midst of revolutionary and very rapid changes; and (2) high-throughput screening cannot be considered in isolation from other aspects of drug discovery. These considerations will shape the long-term future of high-throughput screening. For the present, however, momentum from the existing drug discovery paradigm, with its goals and limitations, will continue to have major influence over the nature of products and services. Indeed, the pharmaceutical industry is virtually forced to catch its breath and assimilate the paradigm governed by optimizing the random screening of large libraries to generate hits,

before it can move on the incorporate changes reflecting the integration of downstream activities into primary screens.

Pharmaceutical companies are forced to deal with several major issues that determine the scope and nature of their high-throughput screening programs. First, quantities of compounds in company libraries are often quite small. Depletion of libraries is highly problematic both because replacement is costly and in many instances extremely difficult to accomplish. Therefore, companies aim to maximize quantities of compounds in libraries and to minimize the quantities used in any given screen. A second key issue relates to the diversity of technological choices that must be made. Reduction of compound usage requires reduction of assay volumes, and such reduction can be accomplished either by reducing the size of microplate wells in the current assay paradigm or by adopting new-paradigm microfluidic and other alternative approaches. Miniaturization through high-density microtiter plates implies a shift to homogeneous assays in order to avoid the difficulties and, perhaps, the impossibility of manipulating microliter or smaller quantities of fluids for separation or washing operations. Platform companies in high-throughput screening provide instrumentation together with, in many instances, reagents and services. Business and product strategies are highly variable with areas of similarity.

The field of high-throughput screening has generated several types of strategic alliances. The technology access agreement is a common mechanism whereby a large company gets to use a new technology and participate in its late-stage development. For that privilege, the pre-commercial stage partner tries to obtain up-front payments, research and development payments, milestone payments, and royalty payments for resulting products. Many collaborations of this sort have indeed provided all or most of these kinds of payments.

The market analysis section of the report contains a series of tables with estimates of current revenues and future projections for high-throughput screening products and services as a whole, and for various subsegments as well. Current U.S. revenues for high-throughput screening instruments, consumables and services in 1998 totaled an estimated $0.61 billion, increasing by 24% to $0.76 billion in 1999. Projected revenues for 2003 total $1.2 billion, reflecting a 29% average growth rate for the period 1999 to 2003. Worldwide correlates of these estimates show $1.45 billion for 1998 with lower than domestic growth (16%) for 1999 to $1.68 million. The projected total for 2003 is $3.89 billion, reflecting a 28% average growth rate for the 2000 to 2003 period. These estimates and projections are broken down further into

instrument, consumable, and service segments. Instruments are further subdivided into workstations and modules, while consumables are divided into reagents and plasticware.

The overall pattern for the industry serving pharmaceutical high-throughput screening is one of steady growth at rates exceeding pharmaceutical sales growth rates by a considerable margin. Growth rates for high-throughput screening exceed even rates of growth for pharmaceutical research and development. Even these high growth rates for screening lag behind the actual needs of the industry. Growth in high-throughput screening product and service revenues will be constrained by caps placed on pharmaceutical research and development expenditures in order to maintain acceptable profit margins. The revenue growth rates reflect a balance between burgeoning opportunity and the realities of spending constraints. The pharmaceutical industry is challenged to increase its output of innovative new drugs while maintaining profit levels that are acceptable to the investment community.

Providers of products and services for high-throughput screening will increasingly be challenged to demonstrate the cost-benefit perspectives of their technology advances. These demonstrations must, when possible, apply to the broader drug discovery process. In other words, it may no longer be sufficient to provide increased throughput for screening while doing nothing to affect downstream bottlenecks in later-stage screening. Alternatively, it may no longer be sufficient to provide high-throughput screening solutions that fail to effectively interface with compound storage and retrieval systems. Adding value, will increasingly reflect broadening the level of integration of technologies with the overall discovery process.

1.7 The Future of High-Throughput Screening

The future of high-throughput screening will be determined to a large extent by the level of funding committed to that activity by pharmaceutical companies. The levels of funding will be determined by a complex equation for optimization of the entire drug discovery process. The weighting given to the various inputs to that equation will differ among companies depending on their cultures, strategies, and the influence borne by one or another faction. A key element in such considerations is that the discovery task has shifted somewhat during the past few years from simply identifying promising leads to the added proviso that dead-end leads should be eliminated from consideration as early in the process as possible. Some inputs to the decision equation are: value received for miniaturization versus resource inputs required; the extent to which new technology provides value that extends beyond the primary screening process; the

information content provided by new technology; and the technology's "homogeneity index". Whether a new technology or instrument is adopted will also depend on the extent to which it provides laboratory integration without destroying flexibility. Pharmaceutical companies are recognizing that their future success is tied to viewing the entire drug discovery process as a single entity.

The extent of integrated automation in the laboratory versus autonomous or semi-autonomous workstations is one of the most important decisions that must be made. The optimal solution for laboratories over the short-term will probably be a mixture of the two. The longer-range future is also likely to see a decreased reliance on high-throughput screening in favor of virtual screening. Some of the most exciting advances in high-throughput screening will no doubt come in the area of cell-based assays. Cellular assays may also be expected to benefit from a number of microfluidic-based efforts.

The demand obviously exists for high-throughput approaches to generating information on metabolic, bioavailability, and toxicity parameters. Progress is starting to be made, and more will undoubtedly follow during the next three years. An intriguing notion, at least from a theoretical point of view, involves the integration of combinatorial chemistry, library storage and acquisition, and screening.

Many believe that success in increasing the number of viable leads will be enhanced through the use of *in silico* structure modeling and design and integrating both historical and predictive information gleaned from the screening process. Success will require a major emphasis on communication and cooperation between functional groups (synthesis, HTS, and medicinal chemistry) combined with the use of highly sophisticated bioinformatics.

In summary, it appears that opportunity exists for great diversity in high-throughput screening technologies and systems. The dominant theme, continual evolution, is driven by economic realities and pulled along by ever-increasing requirements for increased information content. Diversity and flexibility appear necessary, and computers will make order out of the chaos and permit creative new ways of viewing and mining data. Information systems can go far toward integrating the entire drug discovery and development process. Consequently, the modern screener must be capable of operating from multiple perspectives. Screeners must also be fluent

in molecular biology, computer science, robotics, instrumentation and overall process engineering.

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